P30563

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants : Ryozo NAGAI et al.

Group Art Unit : 1611

Appl. No. : 10/598,275

Examiner : Kyle Purdy

I. A. Filed : February 24, 2005

Confirmation No. : 2170

For : MEDICINE CAPABLE OF INHIBITING ACTIVATION OF TRANSCRIPTION FACTOR KLF5

DECLARATION UNDER 37 C.F.R. § 1.132 OF NAOTO ISHIBASHI

Commissioner for Patents
U.S. Patent and Trademark Office
Customer Service Window, Mail Stop **AF**
Randolph Building
401 Dulany Street
Alexandria VA 22314

Sir :

I, the undersigned, Naoto ISHIBASHI, a citizen of Japan, do solemnly declare as follows:

1. That I received a Master's Degree in 1997 from Tokyo University of Science, Graduate School of Pharmaceutical Science. I have been employed by Kowa Company, Ltd. (formerly Kowa Pharmaceutical Co., Ltd.) since April 1997. Throughout my career with Kowa Company, Ltd. (formerly Kowa Pharmaceutical Co., Ltd.), I have been engaged as a research scientist, especially in the field of pharmacology at Pharmacology Department II, Tokyo New Drug Research Laboratories of the company.

2. That I am not one of the inventors of the above-referenced application, but have been involved in the prosecution of the present application to assist the Intellectual Property department of the company as a specialist in the field of pharmacological science.

3. That I have reviewed Appellant's Appeal Brief, filed April 22, 2011, and the Examiner's Answer to the Appeal Brief mailed September 12, 2011.

4. That I have also reviewed the following documents that are indicated to be the "Evidence Relied Upon" in the Examiner Answer:

Marx et al., *Circ. Res.* **90**:703-710, 2002; and

WO 01/80854 A1 to Shidoji et al. as evidenced by the English equivalent US 2005/0250671 A1.

5. That I conducted experiments in order to study the possibility of suppression by (2E,4E,6E,10E)-3,7,11,15-tetramethyl-2,4,6,10,14-hexadecapentanoic acid (hereinafter "NIK-333") against release of several cytokines. In particular, the following Experiments 1 - 4 were carried out to determine inhibition of the following cytokines:

(1) Interleukin (IL)-2;

(2) Interferon (IFN)-γ;

(3) IL-4; and

(4) Tumor Necrosis Factor (TNF)-α

Experiment 1 - Interleukin (IL)-2

20 μg/mL of Concanavalin A (Con A) was added to human peripheral blood mononuclear cells (PBMC), and the cells were cultured in RPMI-1640 medium (pH 7.4) supplemented with 10% FBS, 100 U/mL Penicillin, 100μg/mL Streptomycin, and 2 mmol/L Glutamine to induce production of IL-2. NIK-333 dissolved in dimethyl sulfoxide (DMSO) was added beforehand to the culture, and culturing was continued at 37°C for 16 hours, and then an amount of IL-2 released in the medium was measured by the ELISA method. DMSO at a final concentration of 0.1% was used as a solvent, and an inhibitory rate was calculated based on the amount of released IL-2 of a control group regarded as 100% which was added solely with the solvent. Duplicate experiments were carried out to obtain an average inhibitory rate.

Experiment 2 - Interferon (IFN)-γ

20 μg/mL of Con A was added to human peripheral blood mononuclear cells, and the cells were cultured in RPMI-1640 medium (pH 7.4) supplemented with 10% FBS, 100 U/mL Penicillin, 100μg/mL Streptomycin, and 2 mmol/L Glutamine to induce production of IFN-γ. NIK-333 dissolved in DMSO was added beforehand to the culture, and culturing was continued at 37°C for 16 hours, and then an amount of IFN-γ released in the medium was measured by the ELISA method. DMSO at a final concentration of 0.1% was used as a solvent, and an inhibitory rate was calculated

based on the amount of released IFN-γ of a control group regarded as 100% which was added solely with the solvent. Duplicate experiments were carried out to obtain an average inhibitory rate.

Experiment 3 - IL-4

20 μg/mL of Con A was added to human peripheral blood mononuclear cells, and the cells were cultured in RPMI-1640 medium (pH 7.4) supplemented with 10% FBS, 100 U/mL Penicillin, 100μg/mL Streptomycin, and 2 mmol/L Glutamine to induce production of IL-2. NIK-333 dissolved in DMSO was added beforehand to the culture, and culturing was continued at 37°C for 16 hours, and then an amount of IL-4 released in the medium was measured by the ELISA method. DMSO at a final concentration of 0.1% was used as a solvent, and an inhibitory rate was calculated based on the amount of released IL-4 of a control group regarded as 100% which was added solely with the solvent. Duplicate experiments were carried out to obtain an average inhibitory rate.

Experiment 4 - Tumor Necrosis Factor (TNF)-α

25 ng/mL of Lipopolysaccharide (LPS) was added to human peripheral blood mononuclear cells, and the cells were cultured in AIM-V medium (pH 7.4) to induce production of TNF-α. NIK-333 dissolved in DMSO was added beforehand to the

culture, and culturing was continued at 37°C for 16 hours, and then an amount of TNF-α released in the medium was measured by the ELISA method. DMSO at a final concentration of 0.1% was used as a solvent, and an inhibitory rate was calculated based on the amount of released TNF-α a control group regarded as 100% which was added solely with the solvent. Duplicate experiments were carried out to obtain an average inhibitory rate.

6. That the following results were obtained and are summarized in the following Table 1. The values indicated are average inhibitory rates by NIK-333 against the production of each of the cytokines.

Table 1

	1 nmol/L	10 nmol/L	100 nmol/L	1 μmol/L	10 μmol/L
IL-2	-3%	-4%	-10%	-2%	1%
IFN-γ	1%	4%	7%	-1%	-3%
IL-4	13%	-3%	-4%	-2%	9%
TNF-α	0%	-3%	-2%	-3%	9%

7. That from the results shown in Table 1, it is revealed that NIK-333 does not have inhibitory action against the release of each of the cytokines, induced from human peripheral blood mononuclear cells by Con A or LPS, even at 10 μmol/L. Therefore, the conclusion from Experiments 1 - 4 is that NIK-333 does not have direct suppressing action against the release of the cytokines in the Experiments.

The undersigned further declares that all statements made herein of his own knowledge are true and that all statements made on information and belief are believed to be true; and further, that these statements were made with knowledge that willful false statements and the like so made are punishable by fine or imprisonment or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the above-captioned application or any patent issuing thereon.

10/27/11
Date

Naoto Ishibashi
Naoto ISHIBASHI